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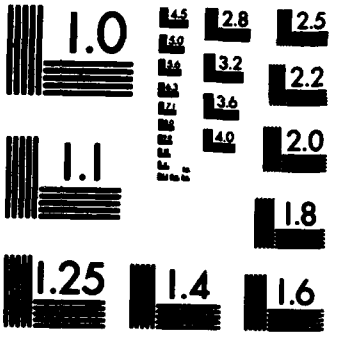

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REPORT DOCUMENTATION PAGE

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1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited
AD-A181 474	5. MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR-TR- 87-0723

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6a. NAME OF PERFORMING ORGANIZATION Baylor College of Medicine	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION AFOSR/NL
6c. ADDRESS (City, State, and ZIP Code) One Baylor Plaza Houston, TX 77030		7b. ADDRESS (City, State, and ZIP Code) Building 410 Bolling AFB, DC 20332-6448
8a. NAME OF FUNDING/SPONSORING ORGANIZATION AFOSR	8b. OFFICE SYMBOL (If applicable) NL	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Grant # AFOSR-85-0178
8c. ADDRESS (City, State, and ZIP Code) Building 410 Bolling AFB, DC 20332		10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61103	2312	46	

11. TITLE (Include Security Classification)
Amine neurotransmitter regulation of long-term synaptic plasticity in hippocampus.

12. PERSONAL AUTHOR(S)
Daniel Johnston

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 4/1/86 TO 3/31/87	4/27/87	16

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Norepinephrine, LTP, hippocampus

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The overall goal of this research project is to investigate the mechanisms of long-term synaptic potentiation (LTP) in hippocampus, with particular emphasis on the modulation of LTP by amine neurotransmitters. It was shown previously that norepinephrine (NE) enhances the magnitude, duration, and probability of induction of LTP at mossy fiber synapses. During the second year of this grant, intracellular recording techniques were utilized to explore various cellular hypotheses associated with this NE modulation of LTP. It was shown that the injection of cyclic AMP (cAMP) into the postsynaptic neuron could mimic the action of NE in enhancing LTP. This very important series of experiments has led to the current working hypothesis that NE modulates LTP by increasing cAMP in the postsynaptic neuron. Additional experiments, which explored possible membrane mechanisms associated with the action of NE on LTP, indicated that NE, through beta-adrenoceptors and cAMP, increases the activity of at least one type of voltage-dependent calcium channel. Other
(Continued on reverse)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
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DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
Unclassified

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19. Abstract, cont.

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AMINE NEUROTRANSMITTER REGULATION OF LONG-TERM SYNAPTIC PLASTICITY IN HIPPOCAMPUS

AFOSR 85-0178

Annual Technical Report

1. Summary

The overall goal of this research project is to investigate the mechanisms of long-term synaptic potentiation (LTP) in hippocampus, with particular emphasis on the modulation of LTP by amine neurotransmitters. It was previously shown that norepinephrine (NE) enhances the magnitude, duration, and probability of induction of LTP at mossy fiber synapses. During the second year of this grant, intracellular recording techniques were utilized to explore various cellular hypotheses associated with this NE modulation of LTP. It was shown that the injection of cyclic AMP into the postsynaptic neuron could mimic the action of NE in enhancing LTP. This very important series of experiments has led to the current working hypothesis that NE modulates LTP by increasing cyclic AMP in the postsynaptic neuron. Additional experiments, which explored possible membrane mechanisms associated with the action of NE on LTP, indicated that NE, through beta-adrenoceptors and cyclic AMP, increases the activity of at least one type of voltage-dependent calcium channel. Other work associated with this grant involved the development of a preparation of mossy fiber synaptosomes for electrophysiological analysis and computer models for simulation of neural networks comprised of realistically represented hippocampal neurons.

2. Research Objectives

The research objectives for the funding period, 1 April 1986 - 31 March 1987, were as follows:

a) Test the hypothesis that the NE enhancement of LTP is mediated in the postsynaptic neuron.

b) Investigate multiple types of calcium channels in hippocampal neurons and test the hypothesis that the NE modulation of LTP is mediated through changes in one or more types of voltage-dependent calcium channels.

c) Develop a preparation enriched in mossy fiber synaptosomes for electrophysiological and biochemical analyses of neurotransmitter release.

d) Develop single neuron and multi-neuron computer models.

3. Status of Research

a) Test the hypothesis that the NE enhancement of LTP is mediated in the postsynaptic neuron. One prediction of this hypothesis is that the previously demonstrated blockade of LTP by propranolol should be overcome by the postsynaptic injection of cyclic AMP. In other words, if the

enhancement of LTP by the endogenous release of NE is mediated by a rise in cyclic AMP in the postsynaptic neuron, then the propranolol inhibition of LTP occurs through the block of beta-adrenoceptors on the postsynaptic neuron. Furthermore, such a block by propranolol should be overcome by artificially raising cyclic AMP in the postsynaptic neuron. We tested this prediction of the above hypothesis using the following experimental protocol.

Three groups of cells were compared. In two groups of cells, the intracellular micropipette contained potassium acetate plus 8-bromo-cyclic AMP (8-br-cAMP), and for one of these groups, a high frequency stimulus train was given to the mossy fibers to elicit LTP. In the third group, the micropipette contained the inactive metabolite of cyclic AMP, 5'AMP, and this group also received high frequency stimulation of the mossy fibers. We found that only the group of cells injected with 8-br-cAMP and which received a high frequency stimulus train displayed LTP (Fig. 1). LTP was determined under voltage clamp conditions in which the conductance was calculated from current measurements at 5 ms and 17 ms after the stimulus. The measurement at 17 ms represents primarily the feed-forward and recurrent inhibitory currents, whereas the early measurement reflects both excitatory and inhibitory currents. We found that there was an increase in the currents measured at 5 ms, but no change in the currents measured at 17 ms. This suggests that there was no change in the inhibitory conductance associated with LTP and that the increase in the conductance measured at 5 ms represented an increase in the mossy fiber evoked excitatory conductance. These results also confirmed earlier findings, which suggested that an increase in cAMP by itself does not produce LTP. However, an increase in cAMP in conjunction with high frequency stimulation will increase both the magnitude and the probability of induction of LTP.

In an attempt to determine possible mechanisms associated with the cAMP enhancement of LTP, we measured the membrane depolarization during the high frequency stimulus train in two of the groups of cells--the 8-br-cAMP group, and the 5'-AMP group. We found that there was a significantly greater and more prolonged depolarization during the train in the 8-br-cAMP group (see Fig. 2). These results suggest that the mechanism of action of NE and 8-br-cAMP is to increase the amount of depolarization during the stimulus train.

A possible mechanism for the enhanced depolarization can be proposed based on results given below. We propose that NE, through beta-adrenoceptors and increased cAMP, enhances the activity of voltage-dependent calcium channels. This enhanced activity of calcium channels will lead to a greater depolarization during the stimulus train and, ultimately, greater influx of calcium. It has been proposed by others that calcium influx is the critical event leading to LTP. Our current working hypothesis, therefore, is in keeping with the idea that greater postsynaptic Ca influx leads to greater LTP.

b) Investigate multiple types of calcium channels in hippocampal neurons and test the hypothesis that the NE modulation of LTP is mediated through changes in one or more types of voltage-dependent calcium channels. In year one of this grant, we explored the action of NE on whole-cell calcium currents measured in acutely-exposed granule cells from hippocampal slices. In year two of this grant, we extended this investigation to the

level of single calcium channels. It has been proposed by others that there are multiple types of calcium channels in neurons—at least two and perhaps three. Our first goal was to explore the possibility that there are multiple types of calcium channels in hippocampal neurons, and second, to determine which of the multiple types of channels was modulated by NE.

Based solely on single channel conductances, we have identified at least three types of calcium channels. Using isotonic barium chloride in the recording pipette and cell-attached recordings, we have identified channels with conductance levels of approximately 8 ps, 14 ps, and 27 ps (see Fig. 3). These different conductance levels could be different states of the same channel or distinctly different channels. This is a topic of some controversey in the literature, and we do not as yet have data to distinguish between these two possibilities.

Nevertheless, we explored the action of NE, isoproterenol, and 8-br-cAMP on the most prominent conductance level observed in our experiments, the 14 ps channel. We have found that the activity of the 14 ps channel, as measured by fractional open time, is increased by the whole-cell application of either the NE, isoproterenol, or 8-br-cAMP (Figs. 4 and 5). We found that these agents produced no change in single channel conductance (Fig. 5) but increase either the probability of opening of the channels or the number of channels. This is the first demonstration of an enhancement in activity of calcium channels in brain, although similar results have been obtained in heart muscle. Several questions remain unanswered, such as possible effects of NE on channel kinetics and whether or not the 8 ps and 27 ps channels are also affected by NE. We also need to test the effects of NE on pyramidal cells. Although preliminary experiments indicate no qualitative differences (that is, NE appears to enhance calcium channels on pyramidal neurons as well as granule neurons), there may be quantitative differences in the distribution of the three channel types and/or effects of NE.

c) Develop a preparation enriched in mossy fiber synaptosomes for electrophysiological and biochemical analyses of neurotransmitter release. In collaboration with Drs. David Terrian (USAFSAM) and Warren Strittmatter, we have been developing a preparation enriched in mossy fiber synaptosomes from rat hippocampus for electrophysiological and biochemical analysis of neurotransmitter release. The following progress has been made:

(1) We have successfully recorded single channels from mossy fiber synaptosomes. The types of channels observed and their properties have not yet been determined. The main problem associated with these electrophysiological studies is the difficulty in identifying the mossy fiber synaptosomes in our recording chamber. We are currently exploring various fluorescent techniques that should enable us to distinguish the mossy fiber synaptosomes from other cellular debris.

(2) Using a fluorometric assay, we have identified a depolarization and calcium-dependent component of glutamate release from the synaptosomes. We have also shown that this calcium-dependent component is enhanced by phorbol esters at a step subsequent to calcium entry (see Fig. 6). We are currently working to improve the sensitivity, reliability, and quantifiability of the assay.

(3) Dr. David Terrian and Dr. Michael Rea (USAFSAM) have developed a radioimmunoassay for dynorphin. They have shown that the preparation is enriched in dynorphin and that dynorphin is released from the synaptosomes in a calcium-dependent fashion. This assay is also undergoing some refinements.

(4) We have measured zinc in various fractions of the preparation and have shown that the preparation we are using for electrophysiological and biochemical analyses is enriched in zinc.

Progress on this supplemental project has been slow but steady and encouraging. If this preparation can be successfully developed such that both the electrophysiological and biochemical analyses of transmitter release can be investigated in a single class of synaptic terminals, then the scientific payoff should be significant. We continue to be very optimistic.

d) Develop single neuron and multi-neuron computer models. We are continuing the development of a set of computer programs that simulate the electrophysiological characteristics of single and groups of hippocampal pyramidal neurons. Our program development takes place primarily during the summer months, when we hire computer science students from Rice. Last summer, developmental work was done on a program called MUNCH that simulates the electrophysiological activity of a single neuron. We also initiated the development of a program called NETWORK, which simulates the behavior of groups of synaptically interconnected neurons. This summer, we hope to use NETWORK for some simple simulations of hippocampal activity and evaluate what our next stage of development should be. We also hope to incorporate more electrophysiological data into both MUNCH and NETWORK to make the simulations as realistic as possible.

4. Publications

Hopkins, W.F. and Johnston, D. Noradrenergic modulation of synaptic plasticity in the hippocampus. In: Developmental Neurophysiology, Kellaway, P. and Purpura, D.P., (eds.), Johns Hopkins Univ. Press: Baltimore, (in press)

Griffith, W.H., Brown, T.H., and Johnston, D. Voltage-clamp analysis of synaptic inhibition during long-term potentiation in hippocampus. J. Neurophysiol. 55:767-775, 1986.

Johnston, D. and Brown, T.H. Control theory applied to neural networks illuminates synaptic basis of interictal epileptiform activity. In: Basic Mechanisms of the Epilepsies: Molecular and Cellular Approaches. Delgado-Escueta, A.V., Ward, A.A., Jr., Woodbury, D.M., and Porter, R.J. (eds.), Raven Press: New York, 1986, pp. 263-274.

Johnston, D., Rutecki, P. A., and Lebeda, F. J. Synaptic events underlying spontaneous and evoked paroxysmal discharges in hippocampal neurons. In: Excitatory Amino Acids and Epilepsy. Schwarcz, R. and Ari, Y. (eds.), Plenum Publishing Corp: New York, 1986, pp. 391-400.

Johnston, D., Hopkins, W. F., and Gray, R. Cellular mechanisms of noradrenergic enhancement of long-term synaptic potentiation in hippocampus. NIDA Research Monograph Series. (in press).

Johnston, D., Hopkins, W. F., and Gray, R. Noradrenergic enhancement of long-term synaptic potentiation. In: Synaptic Potentiation in the Brain: A Critical Analysis. Landfield, P. W. and Deadwyler, S. (eds.), Alan R. Liss: New York, 1987, (in press).

Gray, R. and Johnston, D. Noradrenaline and beta-adrenoceptor agonists increase the activity of voltage-dependent calcium channels in hippocampal neurones. Nature (in press).

Rutecki, P. A., Lebeda, F. J., and Johnston, D. 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. J. Neurophysiol. (in press).

Hopkins, W. F. and Johnston, D. Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. J. Neurophysiol. (submitted).

Abstracts

Gray, R. and Johnston, D. Multiple types of calcium channels in acutely-exposed neurons from adult hippocampus. Biophys. J. 49:432a, 1986.

Hopkins, W.F. and Johnston, D. Noradrenergic enhancement of long-term potentiation in disinhibited hippocampal slices. Soc. Neurosci. Abstr. 12:508, 1986.

Gray, R. and Johnston, D. Multiple types of calcium channels in acutely exposed neurons from the adult guinea pig hippocampus. J. Gen. Physiol. 88:25a-26a, 1986.

Johnston, D., Lebeda, F.J., Barber, S.O., Carnevale, N.T., and Gray, R. Functional reconstruction of hippocampal neurons. Presented at the Fifth Annual Conference on Biomedical Engineering Research in Houston, March 12-14, 1987.

5. Professional Personnel Associated With the Research Project

Daniel Johnston
Frank J. Lebeda
Richard A. Gray
William F. Hopkins
Stan Barber
Judy Walker
Richmond Ansah-Yiadom

6. Interactions

1986

Apr. 4	Gave lecture at the University of Texas Health Science Center in Dallas.
Apr. 6-10	Stan Barber to Masscomp Users' Society meeting in Boston.
Apr. 23-24	Gave lecture ("Noradrenergic modulation of long-term synaptic potentiation in hippocampus") in Irvine, CA.
May 12-16	Attended Masscomp training course
June 11-13	Attended Neurobehavioral Research Review Subcommittee Meeting in Washington, DC.
June 18	Judy Walker and Richmond Ansah-Yiadom visited Dr. Terrian's laboratory in San Antonio.
June 19-20	Dr. Stephen Smith, Dept. of Molecular and Neurobiology, Howard Hughes Institute & Yale Univ. Med. School. Visited, presented lecture on "Transformation of growth cone motility and structure by intracellular calcium and cyclic AMP."
June 26	Dr. Terrian visited the lab.
Aug. 18-19	Baltimore. Spoke at ASPET-SOT.
Sept. 3-7	Rick Gray presented poster at the Society of General Physiologists meeting, Woods Hole, Massachusetts.
September 10	Dr. Johnston and Richard Gray visited Dr. Terrian's laboratory in San Antonio
Sept. 22-23	Attended NIDA Technical Review Meeting on Neural Adaptation in Response to Intrinsic and Extrinsic Factors: Role in Drug Abuse in Rockville, Maryland.
Oct. 3	Attended Second Annual Symposium on Networks in Brain and Computer Architecture at North Texas State University in Denton.
Oct. 22-25	Attended Neurobehavioral Research Review Committee in Washington, DC
Nov. 9-13	Dr. Johnston, Stephen Williams, and William Hopkins (Nov 9-14) attended the Society for Neuroscience meeting in Washington, DC

1987

Feb. 11-13	Attended Neurobehavioral Research Review Committee meeting in Washington, DC

Feb. 18-19	Richmond Ansah-Yiadom to San Antonio to visit Dr. Terrian's laboratory
Feb. 23-24	Attended Biophysical Society meeting in New Orleans
Feb. 27	Drs. David Terrian and Michael Rea visited the laboratory.
Mar. 12-13	Gave lecture (Functional reconstruction of hippocampal neurons) at Biomedical Engineering Research Conference in Houston.
Mar. 27	Gave lecture (Cellular mechanisms of epilepsy) to MSTP students at Baylor.
Mar. 30-31	Dr. David Terrian visited the laboratory.

7. New Discoveries, Inventions, or Patent Applications

None

FIGURE LEGENDS

Fig. 1, above. The phenomenon of LTP measured under voltage-clamp conditions. A: Mossy fiber synaptic currents (single sweeps) sampled at four different holding potentials before and 15 minutes after high-frequency stimulation. The dashed vertical lines correspond to the fixed latencies (5 and 17 ms following stimulus artifact) at which synaptic currents were measured. B: Current-voltage plots for the cell shown in A. Each data point is the mean of five sweeps. Before tetanic stimulation, the slope conductance for the early current measurement (5 ms) was 12 nS, and the extrapolated reversal potential was -34 mV. At 15 minutes following the high-frequency train, the slope conductance for the early current measurement was 22 nS, and the extrapolated reversal potential was -38 mV. The baseline slope conductance for synaptic current measured at 17 ms was 16 nS and was 17 nS at 15 minutes posttetanus. The apparent reversal potential for this current changed from -81 mV to -76 mV over this time period. This experiment was performed in normal saline, and the recording pipette contained 2 M potassium methylsulphate. This cell had an input resistance of 50 megohms and a resting potential of -55 mV.

Fig. 1, below. Summary data from experiments in which LTP was measured under voltage-clamp conditions with intact inhibition in the presence of propranolol (100 nM). A: Bar graph depicts the percent change in the mossy fiber slope conductance for the three groups of cells (see text). G_s refers to the mossy fiber slope conductance measured 5 msec after the stimulus artifact. Only the cells injected with 8-bromo-cAMP and tetanically stimulated demonstrated a significant increase in G_s as a group. B: Graph indicates the probability of observing a significant increase in G_s for the three groups of cells.

Fig. 2, above. A comparison of the membrane potential during tetanic stimulation (100 Hz, 2 sec) in a cell injected with 8-bromo-cAMP (10 mM, top trace) with a cell injected with 5'-AMP (10 mM, bottom trace). Both cells were kept at -65 mV in current clamp during the train. The duration of the high-frequency train is indicated by the bar under the traces. The cell injected with 8-bromo-cAMP demonstrated LTP while cell injected with 5'-AMP did not. Data were obtained with intact inhibition in the presence of propranolol (100 nM). The cell injected with 8-bromo-cAMP had an input resistance of 42 megohms and a resting potential of -54 mV, and the cell injected with 5'-AMP had an input resistance of 35 megohms and a resting potential of -52 mV. The membrane potential was sampled at 100 Hz to obtain the traces for this figure.

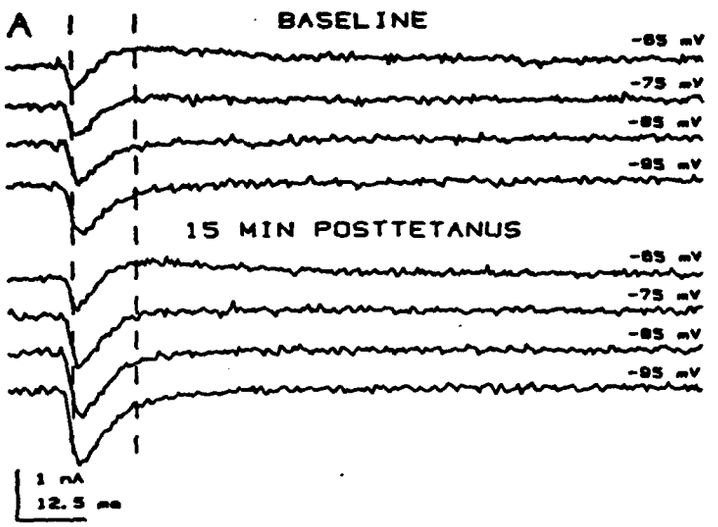
Fig. 2, below. A comparison of parameters of the depolarization during tetanic stimulation in the 8-bromo-cAMP-injected cells that displayed LTP with the 5'-AMP-injected cells that did not. A: The peak amplitude of the depolarization during the high-frequency train was significantly greater in the 8-bromo-injected group. B: The duration of the depolarization during the train was also significantly greater in the 8-bromo-cAMP-injected group.

Fig. 3. Recordings of single calcium channels. Single channel recordings were made from cell-attached patches on granule cells with isotonic $BaCl_2$ in the pipette. Different voltage steps were given to the patches and the current amplitudes plotted vs step voltages. The traces on the left depict examples of the channel openings, and the I-V curves are plotted on the right. Channel openings representing three different slope conductances have been observed in different patches and sometimes in the same patch. Examples of the three conductance levels are given in the Figure.

Fig. 4, a. Application of isoproterenol increases the activity of Ca channels in a cell-attached patch from a granule cell. Patch pipettes contained 96 mM $BaCl_2$; 100 M 3,4-DAP; 1 M TTX; and 10 mM HEPES; the pH was adjusted to 7.35 with TEA-OH. The exposed cells were bathed in 140 mM K-aspartate; 20 mM dextrose, 1 $MgCl_2$; 10 mM EGTA; 10 mM HEPES. The pH was adjusted to 7.4 with KOH. The K-aspartate saline was used to zero the membrane potential across the whole cell (see Nowycky et al.[18]). Voltage jumps were applied to the membrane patch through the patch electrode, and potentials given refer to the patch membrane potential. 1, Consecutive traces of channel activity in control saline in response to step commands from -100 to -10 mV. 2, Channel activity in the same patch 95 s after a 500 ms pressure pulse was applied to a puffer pipette that was located near the cell body and contained K-aspartate saline plus 2 M isoproterenol. 3, Plot of the fraction of time during the command when the current level was more negative than a threshold level (-0.3 pA) before and after application of isoproterenol. The threshold was set at -3 times the standard deviation of the baseline noise calculated from traces in which no channel activity was visible. Each plotted point is the average of three traces, and the horizontal lines indicate the means for all traces before and after drug application. Current traces shown were filtered at 2 kHz (-3 dB, Bessel response) and sampled at 10 kHz. Calibration bar: 2 pA x 10 ms. b. Experiment in which 8-bromo-cAMP was applied in a manner similar to a.

Fig. 5. Single-channel activity at different command voltages before and after isoproterenol. The holding voltage was -100 mV. Patch voltages are relative to the cell potential, which was near zero in K-aspartate saline. Data are from the same patch as that illustrated in Fig. 3a. a, Sample traces are shown for each of four different command voltages. The major current level at each command voltage is indicated by the dashed line. These data were filtered at 1 kHz. b, Same as a, but 400-800 s after a 500 ms pressure pulse was applied to a puffer pipette containing K-aspartate saline plus 2 M isoproterenol. Calibration bar: 1 pA x 10 ms. c, Plot of major single-channel current levels versus patch potential before and after applying isoproterenol. The slope conductance was 14.5 pS and did not change after isoproterenol application. d, Plot of fractional open time versus patch potential before and after isoproterenol.

Fig. 6. Glutamate release from a preparation of synaptosomes enriched in mossy fiber terminals. Glutamate release was measured fluorometrically in response to KCl stimulation. Plot on left is a dose-response curve for the effects of a phorbol ester on the glutamate release that occurs with 20 mM KCl. Plot on right shows glutamate release as a function of KCl with and without Cd. In other experiments, most of the glutamate that is released has been found to be calcium-dependent. Calcium channel blockers such as Co have also been used to demonstrate a calcium-dependent component of release.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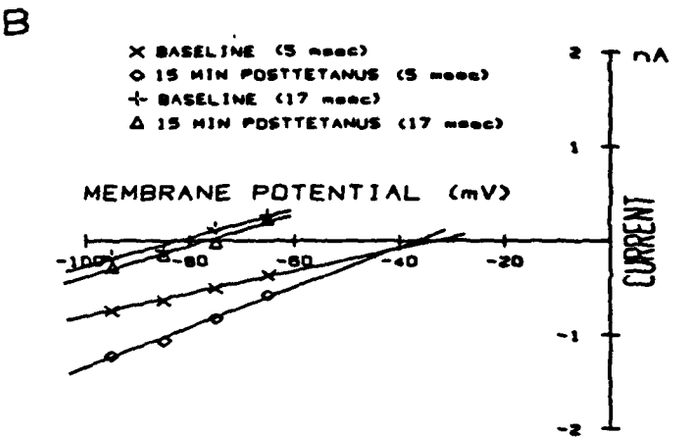
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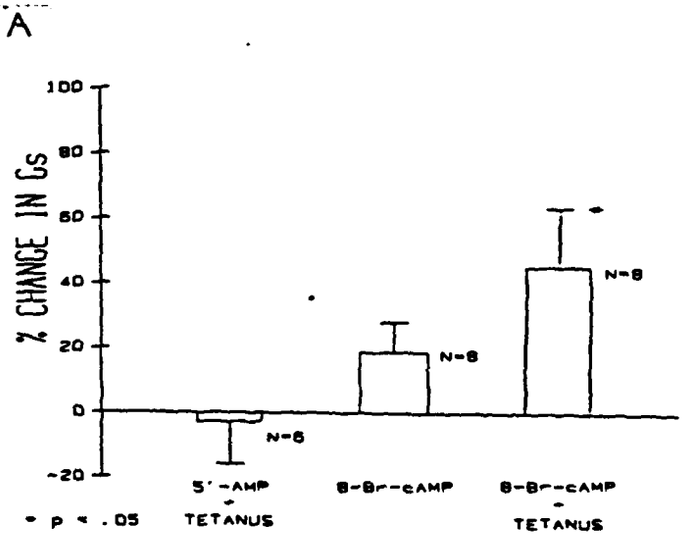
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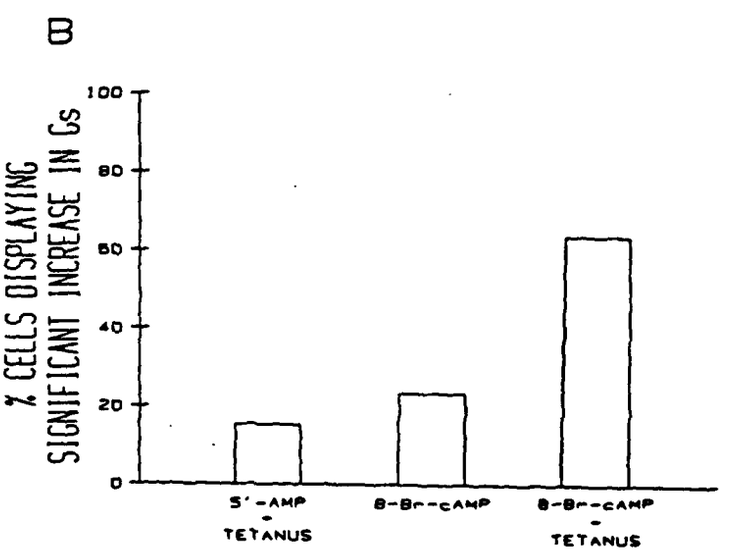
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Fig. 1

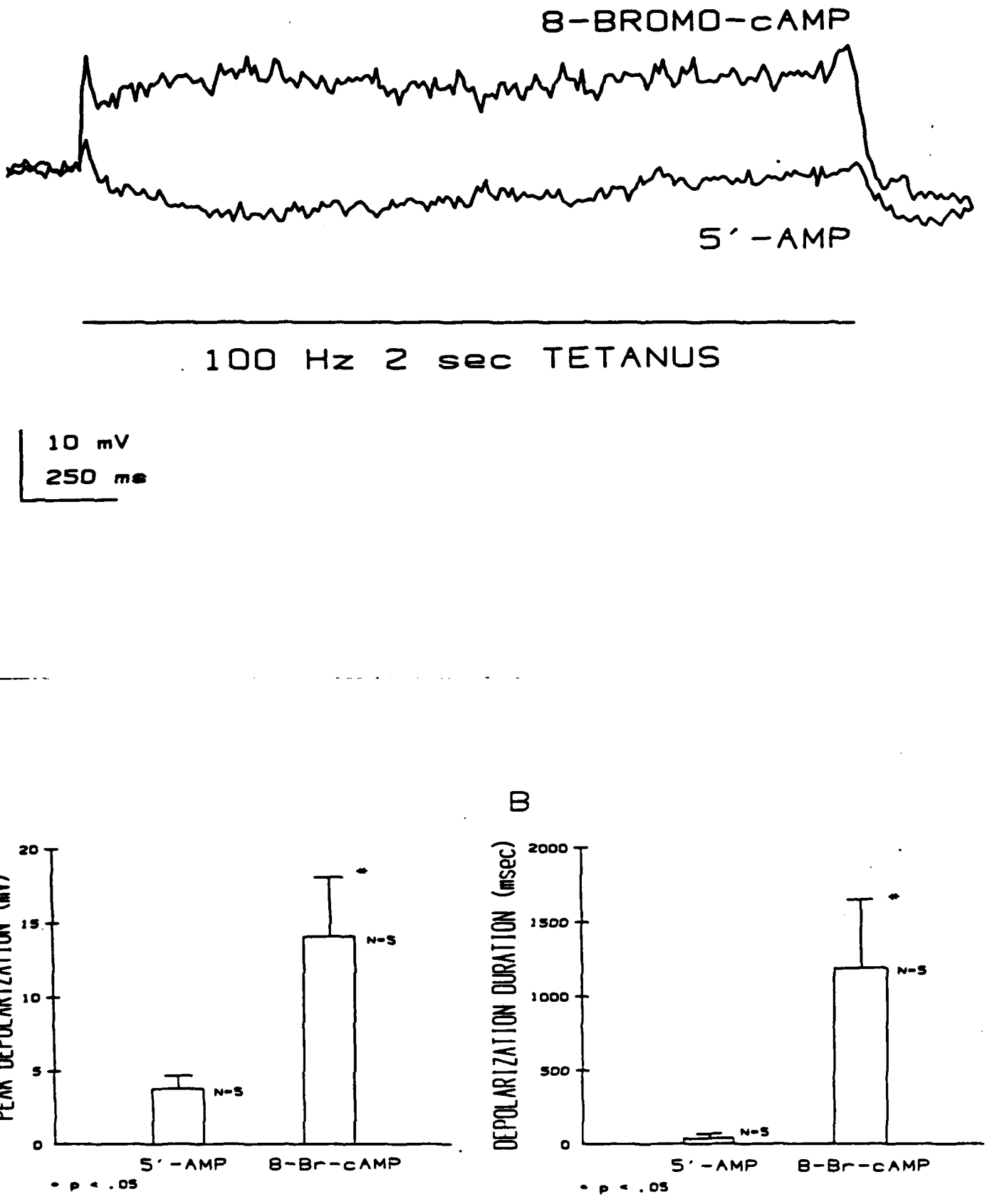
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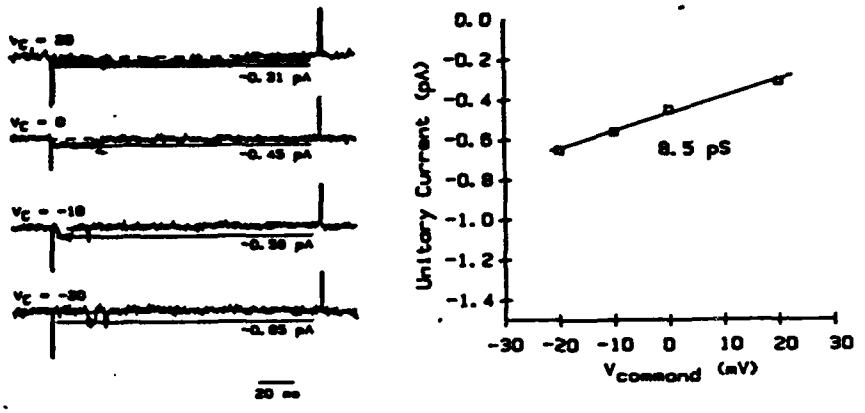
Fig. 2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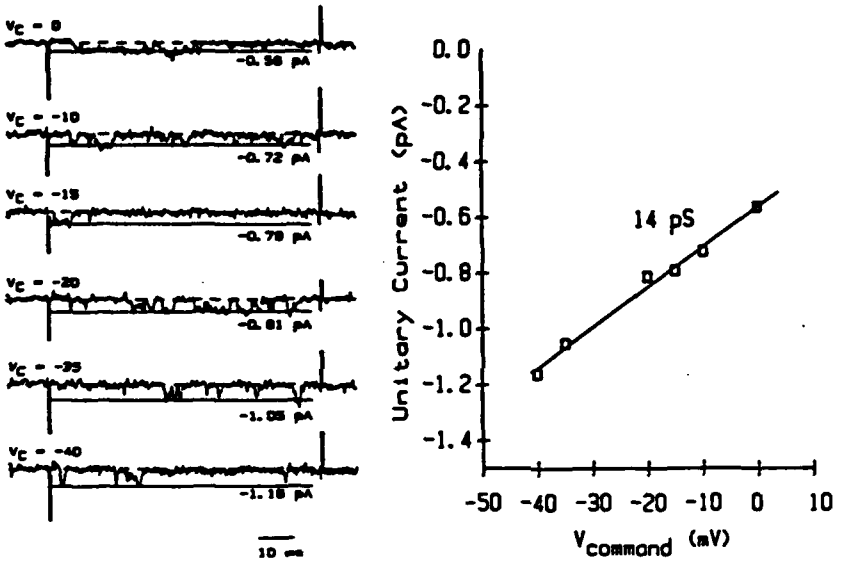
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-1.2
-1.4
-50 -40 -30 -20 -10 0 10
Vcommand (mV)

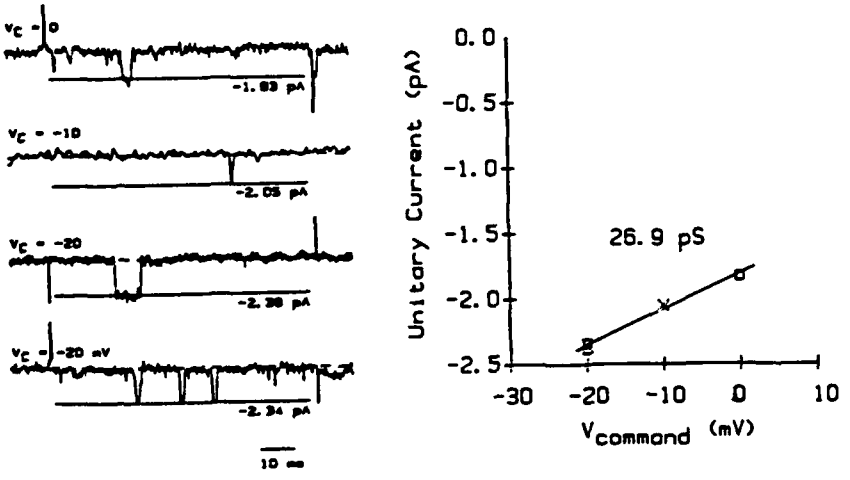

-1.83 pA
-2.05 pA
-2.38 pA
-2.34 pA
10 ms
26.9 pS
Unitary Current (pA)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-30 -20 -10 0 10
Vcommand (mV)

Fig. 3

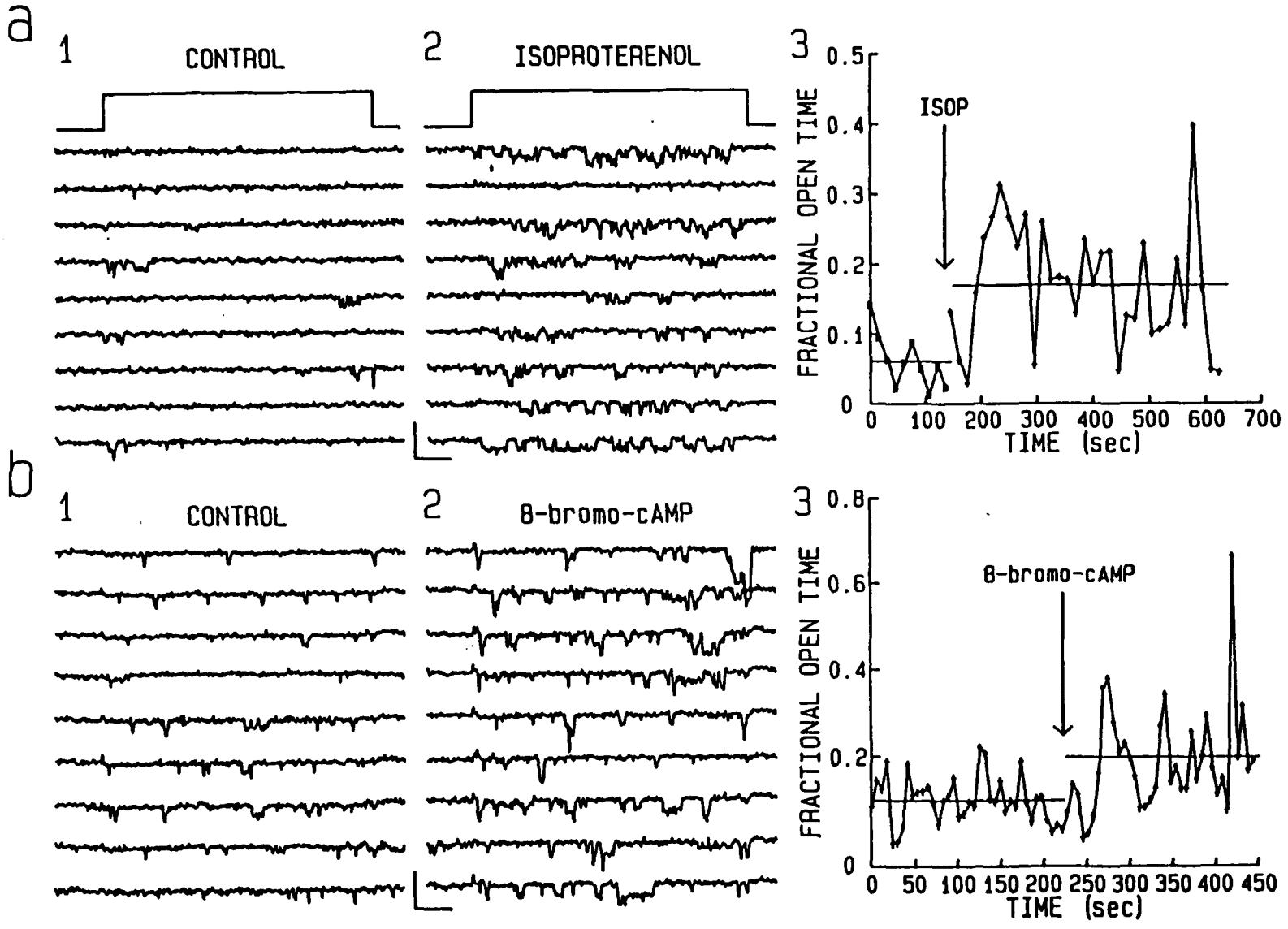

a
1
CONTROL
2
ISOPROTERENOL
3
FRACTIONAL OPEN TIME
ISOP
TIME (sec)
b
1
CONTROL
2
8-bromo-cAMP
3
FRACTIONAL OPEN TIME
8-bromo-cAMP
TIME (sec)

Fig. 4

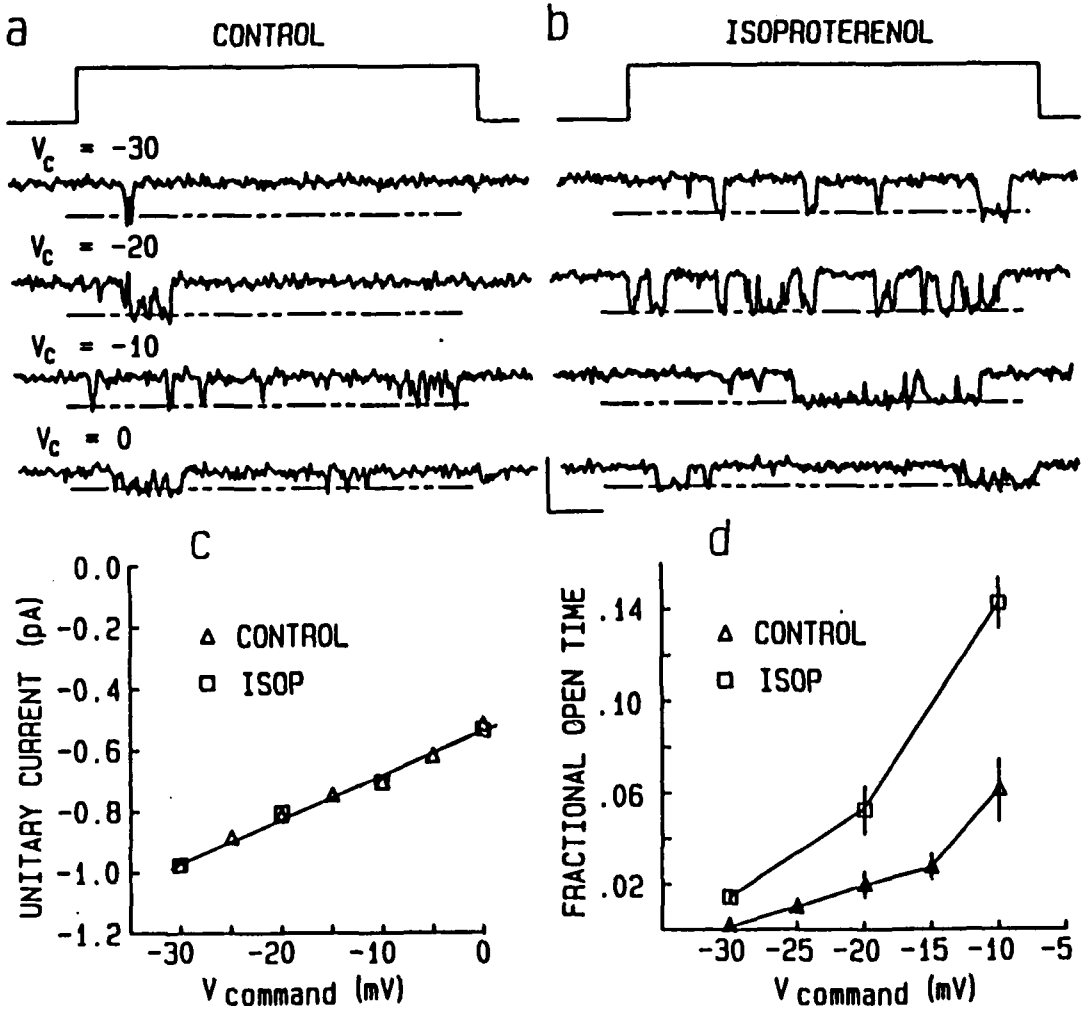
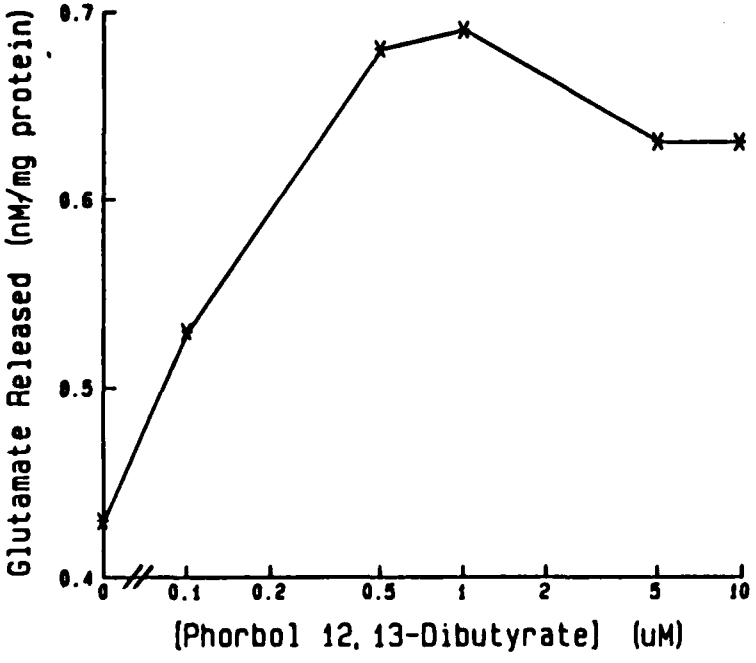

a
CONTROL
b
ISOPROTERENOL
V_c = -30
V_c = -20
V_c = -10
V_c = 0
c
UNITARY CURRENT (pA)
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
CONTROL
ISOP
-30
-20
-10
0
V command (mV)
d
FRACTIONAL OPEN TIME
.14
.10
.06
.02
CONTROL
ISOP
-30
-25
-20
-15
-10
-5
V command (mV)

Fig. 5


Glutamate Released (nM/mg protein)
0.7
0.6
0.5
0.4
0
0.1
0.2
0.5
1
2
5
10
[Phorbol 12, 13-Dibutyrate] (uM)

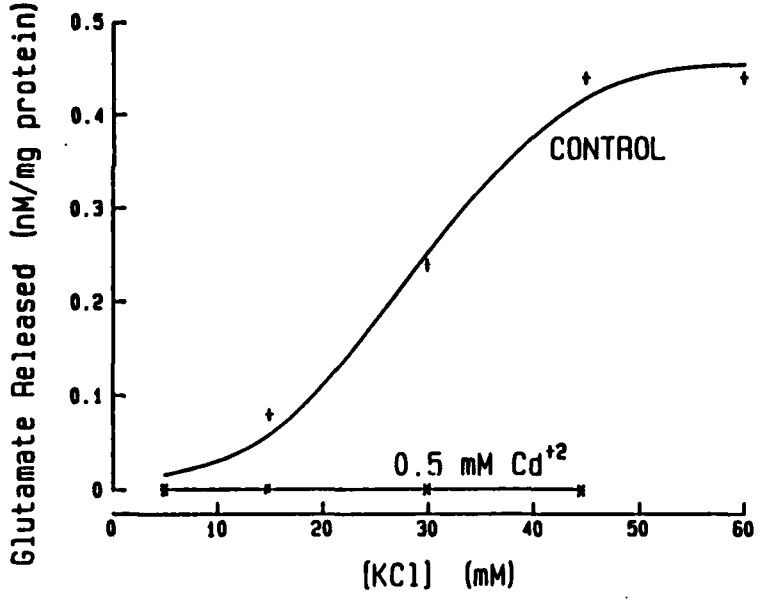

Glutamate Released (nM/mg protein)
0.5
0.4
0.3
0.2
0.1
0
CONTROL
0.5 mM Cd+2
0
10
20
30
40
50
60
[KCl] (mM)

Fig. 6

END

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DTIC